CLAIMS

We claim:

1. A method for the recombination of nucleic acid constructs, comprising:

a) providing:

i) a first nucleic acid construct comprising, in operable order, an origin of replication, a first sequence-specific recombinase target site, and a nucleic acid of interest;

ii) a second nucleic acid construct comprising, in operable order, an origin of replication, a regulatory element and a second sequence-specific recombinase target site adjacent to and downstream from said regulatory element; and

iii) a site-specific recombinase;

b) contacting said first and said second nucleic acid constructs with said site-specific recombinase under conditions such that said first and second nucleic acid constructs are recombined to form a third nucleic acid construct, wherein said nucleic acid of interest is operably linked to said regulatory element.

2. The method of Claim 1, wherein said regulatory element comprises a promoter element.

3. The method of Claim 1, wherein said regulatory element comprises a fusion peptide.

4. The method of Claim 3, wherein said fusion peptide comprises an affinity domain.

5. The method of Claim 3, wherein said fusion peptide comprises an epitope tag.

6. The method of Claim 1, wherein said nucleic acid of interest comprises a gene.

7. The method of Claim 1, wherein said first nucleic acid construct further comprises a selectable marker.

8. The method of Claim 1, wherein said second nucleic acid construct further comprises a selectable marker.

9. The method of Claim 1, wherein said first nucleic acid construct further comprises a prokaryotic termination sequence.

10. The method of Claim 1, wherein said first nucleic acid construct further comprises a eukaryotic polyadenylation sequence.

11. The method of Claim 1, wherein said first nucleic acid construct further comprises a conditional origin of replication.

12. The method of Claim 1, wherein said first sequence-specific recombinase target site is selected from the group consisting of *lox*P, *lox*P2, *lox*P3, *lox*P23, *lox*P511, *lox*B, *lox*C2, *lox*L, *lox*R, *lox*Δ86, *lox*Δ117, *frt*, *dif*, *lox*H and *att*.

13. The method of Claim 1, wherein said second sequence-specific recombinase target site is selected from the group consisting of *lox*P, *lox*P2, *lox*P3, *lox*P23, *lox*P511, *lox*B, *lox*C2, *lox*L, *lox*R, *lox*Δ86, *lox*Δ117, *frt*, *dif*, *lox*H and *att*.

14. The method of Claim 1, wherein said first nucleic acid construct further comprises a polylinker.

15. The method of Claim 1, wherein said contacting said first and said second nucleic acid constructs with said site-specific recombinase comprises introducing said first and said second nucleic acid constructs into a host cell under conditions such that said third nucleic acid construct is capable of replicating in said host cell.

16. The method of Claim 15, wherein said site-specific recombinase is encoded by said host cell.

17. The method of Claim 1, wherein said first nucleic acid construct further comprises a third sequence-specific recombinase target site and said second nucleic acid constructs further comprises a fourth sequence-specific recombinase target site.

18. The method of Claim 17, wherein said first sequence-specific recombinase target site and said third sequence-specific recombinase target site in said first nucleic acid construct are located on opposite sides of said nucleic acid of interest.

19. The method of Claim 17, wherein in said third and fourth sequence-specific recombinase target sites are selected from the group consisting of RS sites and Res sites.

20. The method of Claim 1, wherein said first nucleic acid construct further comprises a third sequence-specific recombinase target site and said second nucleic acid constructs further comprises a fourth sequence-specific recombinase target site, wherein the method further comprises providing a second site-specific recombinase and step c) contacting said third nucleic acid construct with said second site-specific

recombinase under conditions such that said third nucleic acid construct is recombined to form a fourth and a fifth nucleic acid construct.

21. A recombined nucleic acid construct prepared according to the method of Claim 1.

22. A method for the recombination of nucleic acid constructs, comprising:

a) providing:

i) a vector;

ii) a linear nucleic acid molecule comprising a sequence complementary to at least a portion of said vector; and

iii) an *E. coli* host cell, wherein said host cell comprises an endogenous recombination system, a loss of function *rec* mutation, a suppressor, and a loss of function endogenous restriction modification system mutation; and

b) introducing said vector and said linear nucleic acid molecule into said host cell under conditions such that said linear nucleic acid molecule and said vector are recombined to form a recombinant nucleic acid construct.

23. The method of Claim 22, wherein said loss of function *rec* mutation is selected from the group consisting of *recBC* and *recD*.

24. The method of Claim 22, wherein said suppressor comprises *sbc*.

25. The method of Claim 22, wherein said loss of function endogenous restriction modification system mutation comprises *hsdR*.

26. A method for the cloning of nucleic acid libraries, comprising:

a) providing:

i) a plurality of first nucleic acid constructs comprising, in operable order, an origin of replication, a first sequence-specific recombinase target site, and a nucleic acid member from a nucleic acid library;

ii) a plurality of second nucleic acid constructs comprising, in operable order, an origin of replication, a regulatory element and a second sequence-specific recombinase target site adjacent to and downstream from said regulatory element; and

iii) a site-specific recombinase;

b) contacting said plurality of first and second nucleic acid constructs with said site-specific recombinase under conditions such that said plurality of first and second nucleic acid constructs are recombined to form a plurality of third nucleic acid constructs, wherein said nucleic acid members from said nucleic acid library are operably linked to said regulatory elements.

27. A nucleic acid library prepared according to the method of Claim 26.

28. A method for the directional cloning of a nucleic acid molecule, comprising:

a) providing:

i) first and second portions of a regulatory element;

ii) a first nucleic acid molecule comprising said first portion of said regulatory element; and

iii) a second nucleic acid molecule comprising said second portion of said regulatory element; and

b) combining said first and said second nucleic acid molecules to produce a third nucleic acid molecule under conditions whereby an intact regulatory element is produced from the combination of said first and said second portions of said regulatory element, wherein the presence of said intact regulatory element in said third nucleic acid molecule indicates a direction of

cloning of said first nucleic acid molecule with respect to said second nucleic acid molecule.

29. The method of Claim 28, wherein said regulatory element comprises a *lacO* site.

30. A method for regulated recombination in host cells that constitutively express a recombinase, comprising:

a) providing:

i) a host cell expressing a recombinase;

ii) a first nucleic acid construct comprising an origin of replication, a first site-specific recombinase site, a second site-specific recombinase site that differs in sequence from said first site-specific recombinase site such that said recombinase will not initiate recombination between said first and second site-specific recombinase sites, and a selectable marker gene between said first and second site-specific recombinase sites; and

iii) a second nucleic acid construct comprising an origin of replication, a third site-specific recombinase target site, and a fourth site-specific recombinase target site that differs in sequence from said third site-specific recombinase site such that said recombinase will not initiate recombination between said third and fourth site-specific recombinase sites; and

b) introducing said first and second nucleic acid constructs into said host cell under conditions such that said first and second nucleic acid constructs are recombined.

31. The method of Claim 30, further comprising the step of selecting for a desired recombinant nucleic acid molecule using said selectable marker.

32. The method of Claim 30, wherein said first nucleic acid construct is a Univector.

33. The method of Claim 30, wherein said second nucleic acid construct is a Univector.

34. A host cell expressing a recombinant nucleic acid construct prepared according to the method of Claim 30, wherein said host cell constitutively expresses a recombinase.

35. A method for the recombination of nucleic acid constructs, comprising:

a) providing:

i) a first nucleic acid construct comprising a *lox*H site;

ii) a second nucleic acid construct comprising a *lox*H site; and

iii) a site-specific recombinase; and

b) contacting said first and said second nucleic acid constructs with said site-specific recombinase under conditions such that said first and second nucleic acid constructs are recombined.

36. A recombined nucleic acid construct prepared according to the method of Claim 35.

Add E3

ADD G'

93